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Optic Glomeruli: Biological Circuits that Compute Target Identity

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• 14. ABSTRACT

The aim of this project was to investigate synaptic organization in optic glomeruli of the insect visual system. The research is based on the premise that each glomerulus receives an ensemble of small field neurons from the lobula that encodes a specific low-order visual primitive. The expectation is that more complex parameters of visual space are computed by interactions amongst glomeruli via local interneurons that then transmit this data selectively to premotor channels (descending neurons) that control flight initiation, direction and targeting. The present project focuses on synaptic organization of glomeruli, comparing this arrangement with glomeruli in the antennal lobe system for which there is much data available. The nature of this research requires enormous investment in time using serial section electron microscopy as well as patch clamp recording from local interneurons, the smallest neurons in the fly brain.

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Abstract

The aim of this project was to investigate synaptic organization in optic glomeruli of the insect visual system. The research is based on the premise that each glomerulus receives an ensemble of small field neurons from the lobula that encodes a specific low-order visual primitive. The expectation is that more complex parameters of visual space are computed by interactions amongst glomeruli via local interneurons that then transmit this data selectively to premotor channels (descending neurons) that control flight initiation, direction and targeting. The present project focuses on synaptic organization of glomeruli, comparing this arrangement with glomeruli in the antennal lobe system for which there is much data available. The nature of this research requires enormous investment in time using serial section electron microscopy as well as patch clamp recording from local interneurons, the smallest neurons in the fly brain.

1.0 ELECTRON MICROSCOPY AND CIRCUIT ANALYSIS

Comparison of antennal lobe glomeruli with optic glomeruli. Identification of segmental homology.

Electron microscopical analyses of sensory inputs to antennal lobe glomeruli and optic lobe glomeruli demonstrate close correspondence of organization into functional subunits – the glomeruli themselves – and synaptic organization within them. These features suggest that despite different sensory modalities, a common circuit ground pattern exists in each segment of the central nervous system. Antennal lobe glomeruli receive their inputs from olfactory sensory neurons, the population of which is subdivided into receptor types defined by their tuning to specific airborne ligands, the odorants. This tuning is due to each sensory neuron type defined by its own genetically determined receptor molecule that recognizes a limited cohort of odorant molecules. Converging axons from a subset of functionally identical olfactory sensory neurons provide a "labeled line" that targets one specific glomerulus. Each glomerulus therefore receives the terminals of a set of olfactory sensory neurons encoding a specific "olfactory primitive." There are as many glomeruli as there are molecular types of olfactory sensory neurons, which translate to there being as many glomeruli as olfactory primitives detected by the receptor system as a whole. Insects with very few glomeruli detect very few odorants; insects with hundreds of glomeruli detect hundreds of odorants.

Optic glomeruli are similarly organized and likely reflect a similar principle: the number of glomeruli relates to the number of different visual primitives encoded by the lobula. Each glomerulus receives its input from the lobula via a set of retinotopic columnar neurons, the dendrites of which relate to a specific combination of relays from the retina, lamina, and medulla. Studies of the source of optic glomerular inputs (the lobula) show that medulla relays segregate to discrete levels where they provide presynaptic terminals onto the layered dendrites of lobula outputs (Figs.1, 2).

In the antennal lobe, the reconstruction of an odor, which is a blend of primary odorants, is computed amongst glomeruli by local interneurons that then relay this higher order data to neurons that reach various targets in the brain, including the mushroom bodies. Anatomical studies of optic glomeruli show that local interneurons likewise connect different glomeruli. Recordings from relay neurons from the optic glomerular complex reveal that these encode more specific and elaborate parameters of visual space, such as shapes and colors, than those supplying the optic glomerular complex from the lobula (Strausfeld *et al*., 2007; Mu *et al*., 2012). Current recordings from local interneurons – the smallest cells in the brain – using patch clamp recordings on genetically identified cells, reveal that these encode parameters derived from several glomeruli (see section 2.1., below).

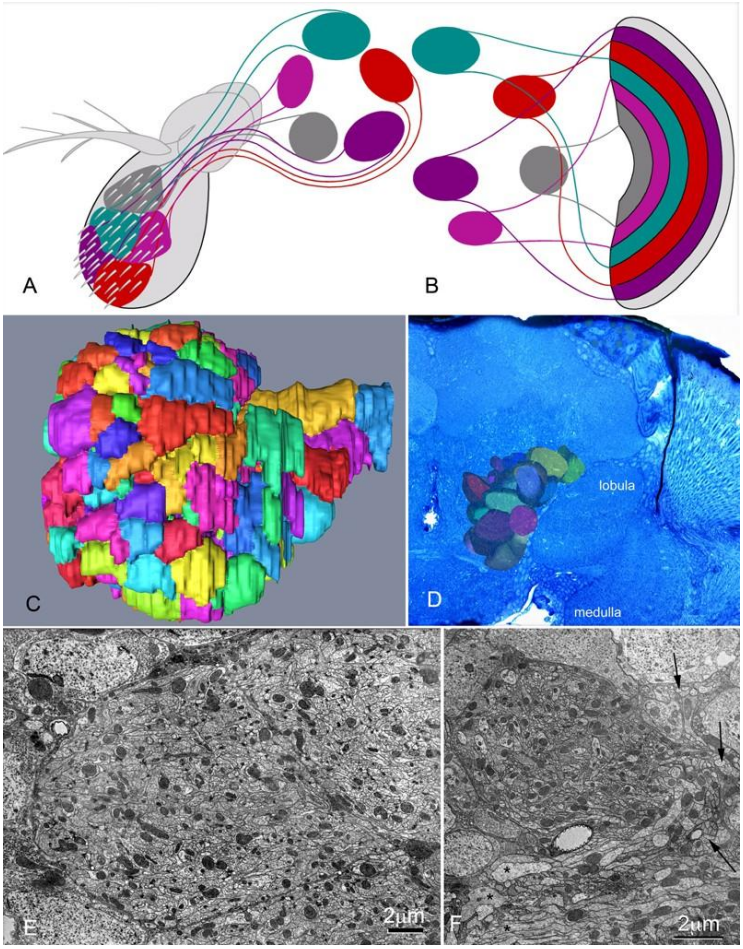

Figure 1 Antennal lobe and optic lobe comparison. A. Sensory input to antennal lobe glomeruli and optic lobe glomeruli demonstrates segmental homology. Antennal lobe glomeruli (A) receive segregated inputs from odortypic antennal receptors. Optic glomeruli (B) receive segregated inputs from the lobula via visuotypic columnar cells. C. 3-dimensional reconstruction of the antennal lobe of *Nasonia vitripennis* was created from 102, 2µm serial histological sections. There are 259 antennal lobe glomeruli that were individually identified, traced and reconstructed using Amira software. This number corresponds to the number of identified odorant receptor genes (301), including 76 pseudogenes (Robertson *et al.* 2010). D. 3-dimensional reconstruction of the optic glomeruli of *Nasonia vitripennis*; it is estimated there are 33 optic glomeruli, located within the lateral protocerebrum. Medulla and lobula optic neuropils are indicated. E, F. Comparisons of transmission electron micrographs of an antennal lobe glomerulus (E) and an optic glomerulus (F) demonstrate their clear morphological similarities. Both glomeruli are discretely identifiable due to the glial border. F. Tracts of neurons from the lobula (arrows) provide input to glomeruli. Three distinct categories of neurons can be identified within the glomerulus; presynaptic profiles with dense cytoplasm, presynaptic profiles with clear cytoplasm, and a population of small round profiles, interpreted as local interneurons. A is from Strausfeld, 2012.

Three-dimensional reconstructions of the antennal lobe and optic glomerular complexes of *Nasonia vitripennis* were generated from over a hundred 2µm-thick serial sections. It is proposed that circuitry of the optic glomeruli follows the same labeled line principle, with

site-specific optic glomeruli each receiving a uniquely defined output from the lobula. Thirty-three optic glomeruli located within the lateral protocerebrum (compared with 27 in *Calliphora*, Strausfeld & Okamura 2007) suggest that this minute hymenopteran reconstructs at least 33 low level visual primitives.

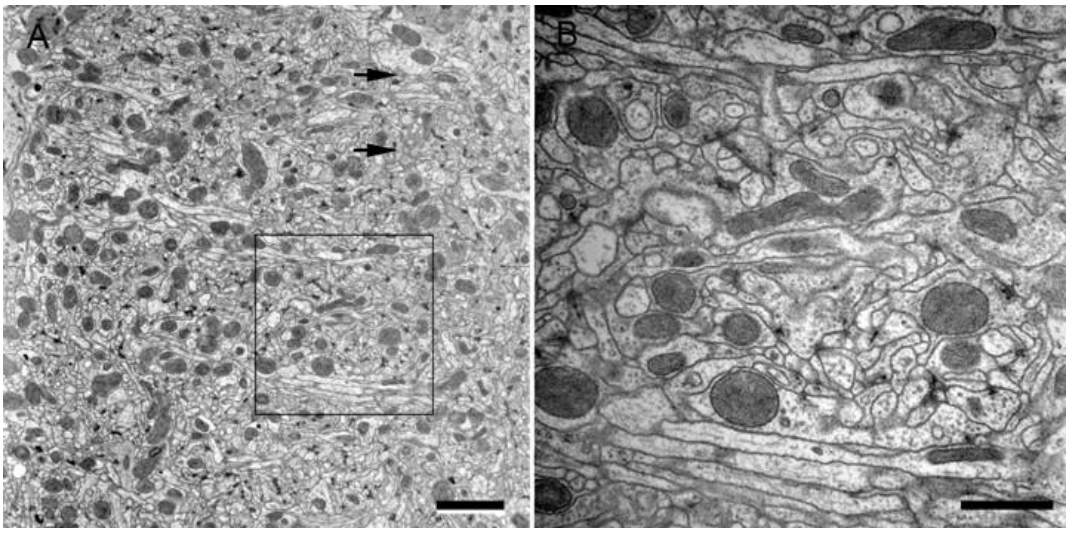

Figure 2 ***Nasonia vitripennis*** **lobula.** The lobula complex is involved in higher processing of both motion detection and color vision pathways. Retinotopic neurons from the medulla sort out by type in the lobula (left, in panel A), each type targeting a specific level of columnar neuron dendrites (in rectangle; shown enlarged in panel B). Axons from these subunits extend to optic glomeruli. (see: Strausfeld & Okamura 2007, Okamura & Strausfeld 2007). Scale bars, A; 2 µm, B; 1µm.

High-resolution studies of optic glomeruli reveal three morphologically distinct populations of profiles that correspond to input neurons, local interneurons, and output neurons. These three distinct categories of neurons can be identified within each glomerulus; presynaptic profiles with dense cytoplasm; pre- and postsynaptic synaptic profiles with clear cytoplasm, and populations of small round profiles, interpreted as local interneurons that are both pre- and postsynaptic (Fig. 3). This type of circuit may encode translational invariance, with separate networks encoding object recognition, object position, and feedback loops that provide information about where such recognition has occurred within the retinotopic system.

Electrophysiological studies are being performed on *Drosophila*, because genetic expression of green fluorescent protein provides identification of cell types in the living fly, which can be then patched. However, our circuitry studies using electron microscopy focus on the far smaller parasitoid *N. vitripennis*. This is because size, or the lack of it, matters. Small is a special advantage for EM work. Fortunately, surveys of the whole brain of *N. vitripennis* (Brown and Strausfeld, in revision) show that it does not depart from the normal ground pattern of brain organization, which means that circuit organization in *Nasonia* conforms to those of large species.

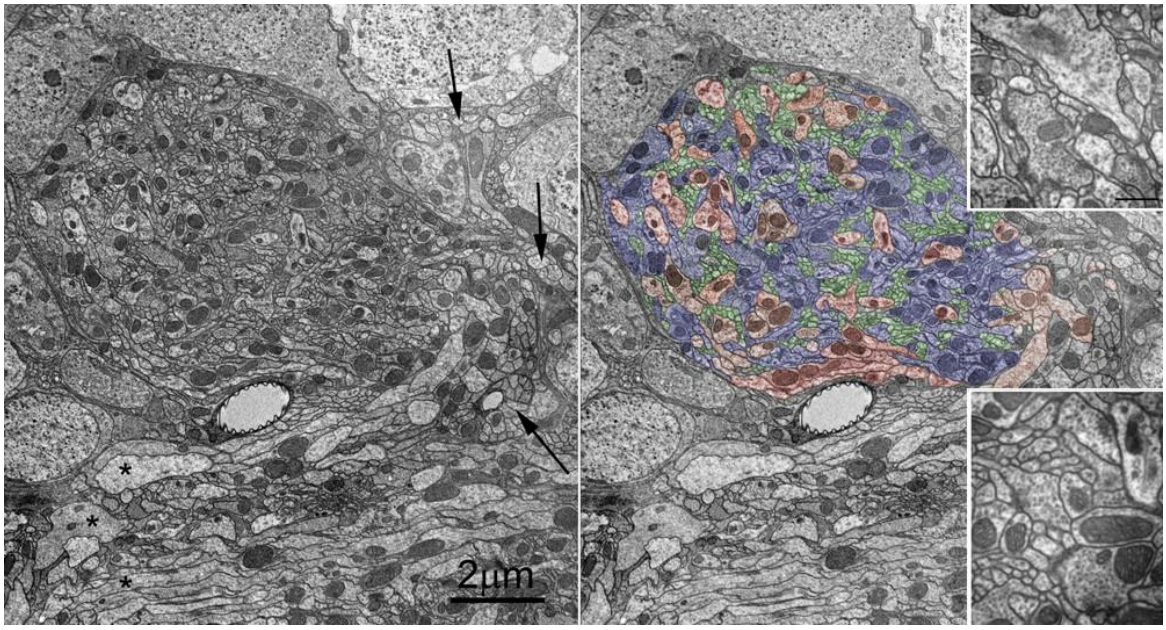

Figure 3. ***N. vitripennis*** **optic glomerulus.** Left panel: Optic glomeruli are discretely identifiable due to their glial borders, and as in the olfactory glomeruli, they have elaborate internal architectures. Tracts of neurons from the lobula (arrows) provide sensory input to site-specific glomeruli. Right panel: Same image, with colors indicating the 3 populations of neurons that characterize a glomerulus; green – local interneurons, purple – presynaptic profiles of lobula output terminals, with dense cytoplasm (see also insets, panel to the right); and orange – presynaptic clear cytoplasm. Local interneurons and projection neurons provide integrative circuitry within and among glomeruli. Optic glomeruli are also supplied by processes from regions of the brain associated with sensory modalities other than vision, suggesting that optic glomeruli are integrative centers provided with information about behavioral decisions. Scale bars inset 0.5µm.

2.0 ELECTROPHYSIOLOGICAL ANALYSIS OF THE OPTIC GLOMERULAR COMPLEX

2.1 Responses of local interneurons.

The results of three years of research on the functional relationship between lobula outputs, the optic glomerular complex, and premotor descending neurons have been published in 2012 (Mu et al., 2012). Here we show an example of a further and deeper analysis of a local interneuron that supports the premise that this class of neurons plays cardinal roles in further reconstructing the visual scene (Fig. 4). Recordings like these, from *Drosophila*, provide further evidence that integration by local neurons in the optic glomerular complex parallels that known from studies of the antennal glomeruli. In both systems, local interneurons reassemble the sensory scene, and it might be speculated that similar circuits amongst sensory domains in thoracic ganglia likewise assemble and encode dynamic changes and fluctuations in mechanosensory space.

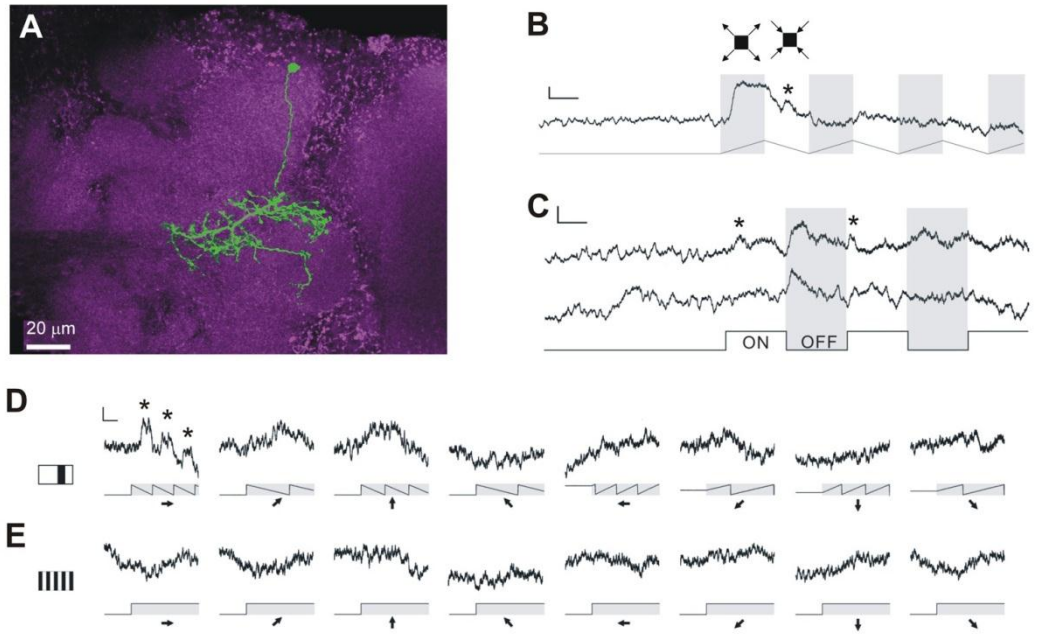

Figure 4. Optic glomerulus local interneuron in *Drosophila*. The neuron links glomeruli receiving inputs from groups of LC neurons. The neuron (panel A) integrates subtle signals and displays strong responses to specific visual stimuli. Panel B: responses to expansion and retraction of a black square. Expansion initiates strong depolarization; contraction initiates a weak depolarization (asterisks). Panel C. The interneuron has stronger response to the "off" signal than "on," as suggested by responses to slow full-field flicker where the size of depolarization initiated by light "off" stimulus is larger than to light "on." Panel D. The interneuron shows directional selectivity to a single bar motion (horizontal motion, front to back) but not to square wave grating motion (panel 1E). Response adaptation during looming stimulus is not obvious for the preferred direction of single bar motion, where sizes of depolarization in three cycles are similar (indicated by asterisks in Fig. 1D). Scale bars: 2 mV/500 ms in B and C; 2 mV/1s in D, E.

2.2 A model descending neuron: testing multimodal integration.

Because green fluorescent protein (GFP) was not exclusively expressed in the giant fiber in our *Drosophila* lines, we have used streptavidin:Cy3 to visualize Giant descending neurons (the "Giant Fiber" GF) after backfilling them with biocytin during intracellular recording. The contralateral giant commissural interneurons connecting the left and right ventrolateral protocerebrum sometimes were also labeled, together with the GF, which is consistent with previous dye backfilled results. All of the visual and mechanical stimuli used alone fail to initiate action potentials in the GF of *Drosophila*. Nevertheless, the GF did show responses (subthreshold depolarization or hyperpolarization) to various unimodal stimuli. Moreover, current injection was able to initiate action potential in those GF. This reluctance to spike reflects a typical characteristic of command neurons mediating rapid behavioral responses, such as GF.

In previous studies, we reported that the GF responds to looming stimulus (Mu et al., 2012). We have further examined GF responses to different types of looming stimuli (Fig. 5). We used three types of looming stimulus, including a black-square expanding on the bright background, a bright-square expanding on the black background, and a chessboard square expanding on the bright background. As reported before, the black- square looming stimulus initiates depolarization response whereas white-square looming and the chessboard-square looming do not. This suggests that using the black-square looming stimulus, the GF is responding to decreasing luminance other than fast expanding edges. When given a continuous looming stimulus, the responses of GF quickly adapted in some flies but not in others. We also tested the GF responses to looming stimuli at different expanding speeds, which did show significant difference between high speed looming and low speed looming.

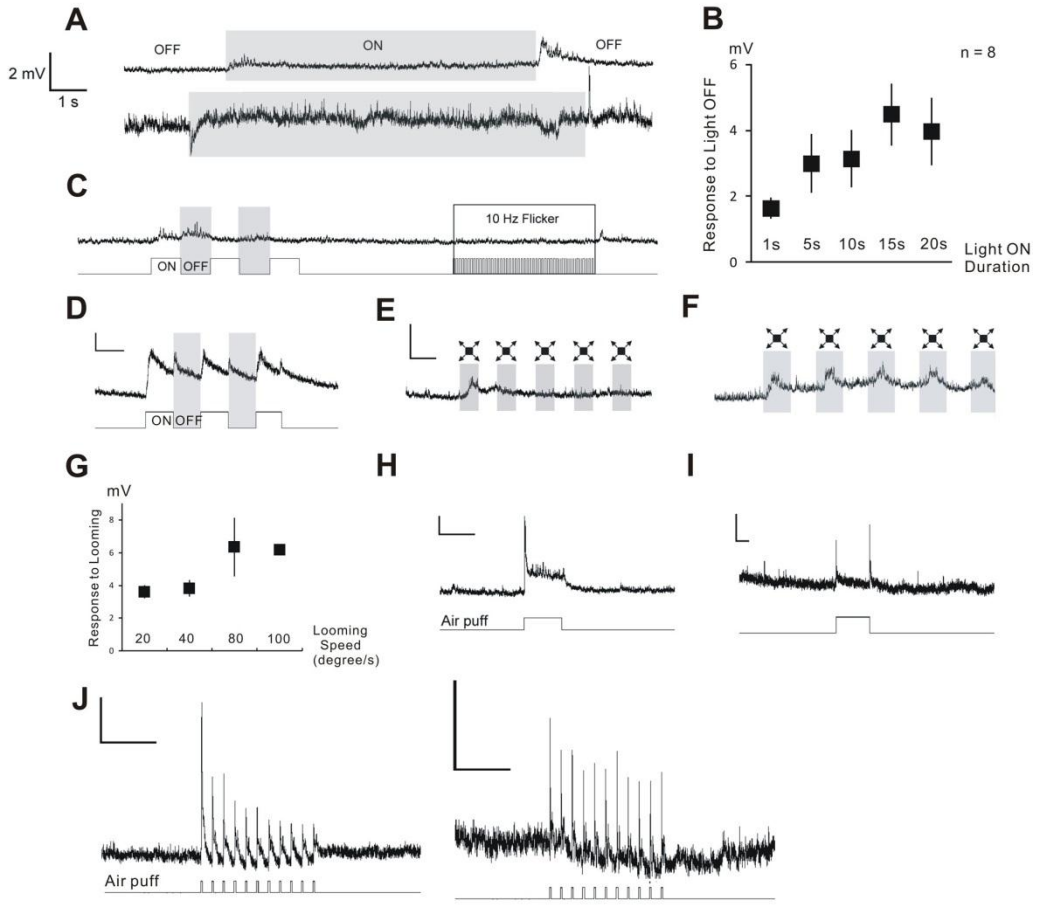

Figure 5. Giant fiber descending neuron response to visual and mechanical stimuli. (A) Light OFF elicits a large EPSP; light ON elicits depolarization or hyperpolarization. Response intensity to light OFF relates to preceding light ON (B). In most of cases using slow flicker (0.5Hz) only the first ON and OFF phases initiate an obvious response (C). 10Hz flicker stimulus initiate no noticeable EPSP or IPSP whereas light OFF following the end of flicker initiates depolarization (C). Black-square looming stimulus initiates depolarization (E, F). Continuous looming stimulus results in fast GF adaptation in some (E) but not other flies (F). GF responses to looming stimuli at different expanding speeds (G) show significant differences between high speed and low speed looming (ANOVA, $p<0.05$, 20^o/s and 40^o/s vs 80^o/s and 100^o/s). Air puff stimuli to the antennae elicit a large EPSP at stimulus start (H) and sometimes at termination (I). Responses following initial EPSP vary: membrane potential can maintain at its depolarization state throughout the whole stimulus (H), or more small EPSPs are elicited than during the resting state. In other examples, the membrane potentials drop close to that of resting (I). The GF variously adapts to a continuous of air puff stimuli (J). All scale bars indicate 2mV/1s.

In *Drosophila*, both looming (approaching object) and light "OFF" can initiate escape behavior. Although it has long been postulated that the GF is responsible for both escape behaviors, recent studies found different motor sequences in those two escape behaviors and imply another neural pathway underlying "looming" initiated escape instead of the GF pathway (Fotowat et al, 2009). Our results did not provide direct evidence supporting the GF's executive role in "looming" initiated escape behavior. However, subthreshold

responses of GF to black-square looming stimulus might contribute to leg and wing extension prior to the final jump movement. Moreover, the mixed responses of GF to varied visual stimulus suggest that underlying neural circuits that provide visual inputs to GF are complex and presynaptic modulation by other modalities might cause varied responses.

Studies on larger flies than *Drosophila* show that the GF responds to mechanical stimuli to the antenna (Bacon and Strausfeld, 1986). Additionally, the GF in *Drosophila* showed responses to courtship song (Tootoonian et al., 2012). We examined how the GF responds to air puff applied to its antennae (Fig. 5). Our results showed that this stimulus elicits a large excitatory postsynaptic potential (EPSP) at the beginning of the stimulus, and, occasionally at the end of stimulus. Responses following the initial large EPSP are varied; sometimes the membrane potential is maintained at the depolarization state through the whole air puff phase; other times there are more small EPSPs emerged during air puff stimulus than during the resting state. In other examples, the membrane potentials fall close to that of the resting state. The GF also shows varied adaptive responses to a continuous of air puff stimuli. However, the size of the EPSP responding to the onset of the first air puff is larger than those of the following air puff stimuli in all of cases.

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Author: Laiyong Mu and Nicholas J. Strausfeld
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Title: Responses to defined visual stimuli by small lobula columnar neurons in *Drosophila melanogaster*.
Author: Laiyong Mu and Nicholas J. Strausfeld
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Title: Responses to defined visual stimuli by small lobula columnar neurons in *Drosophila melanogaster*.
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Poster#: 78.20/GG1

2013 March 3 – March 6, Insect Vision: Cells, Computation, and Behavior, Janelia Farm Conference, Ashburn, VA
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